Analytical method for tebuconazole in water

ECM: EPA MRID No. 50755102. Navarro, F. 2018. Environmental **Reports:**

> Chemistry Method: Validation of the Analytical Method for the Determination of Tebuconazole in Aqueous Matrices by LC-MS/MS. Smithers Viscient Study No.: 14162.6113. Report prepared by Smithers Viscient, Wareham, Massachusetts, and sponsored and submitted by Generic Tebuconazole DCI Task Force, c/o United Phosphorus, Inc., King of Prussia,

Pennsylvania; 59 pages. Final report issued December 20, 2018.

ILV: EPA MRID No. 50768802. Cashmore, A. 2019. Tebuconazole – Independent Laboratory Validation in Water. Study No.: 3202240. Report prepared by Smithers Viscient (ESG) Ltd., North Yorkshire, United

Kingdom, and sponsored and submitted by Generic Tebuconazole DCI Task Force, c/o United Phosphorus, Inc., King of Prussia, Pennsylvania, and Pyxis Regulatory Consulting, Inc., Gig Harbor, Washington; 63 pages. Final report

issued January 30, 2019.

MRIDs 50755102 & 50768802 **Document No.:**

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA (40 CFR

> 160) Good Laboratory Practice (GLP) standards, which are compatible with OECD Principles of GLP (p. 3 of MRID 50755102). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided

(pp. 2-4). An authenticity statement was included with the Quality

Assurance statement.

ILV: The study was conducted in accordance with USEPA FIFRA (40 CFR 160), UK, and OECD GLP standards, except for the water characterization (p. 3; Appendix 3, p. 49 of MRID 50768802). Signed and dated No Data Confidentiality, GLP, Quality Assurance, Authenticity statements were

provided (pp. 2-6; Appendix 3, p. 49).

This analytical method is classified as supplemental. An updated ECM **Classification:**

> should be submitted with directives to rinse glass jars with acetonitrile prior to use due to possible carryover issues. The specificity of the method for surface water was not supported by ECM representative chromatograms.

PC Code: 128997

EFED Final

Signature: Mrun My **Reviewer:** Andrew Shelby,

> **Date:** 3/22/2021 Physical Scientist

CDM/CSS-Lisa Muto, M.S.,

Dynamac JV **Environmental Scientist Reviewers:**

Signature: Les Muto

Date: 05/23/2019

Signature: Mary Samuel Mary Samuel, M.S., **Environmental Scientist**

Date: 05/23/2019

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

The analytical method, Smithers Viscient Laboratory Project No.: 14162.6113, is designed for the quantitative determination of tebuconazole in water at the LOQ of 0.100 µg/L using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water for tebuconazole. The ECM and ILV validated the method using two water matrices; different water for each validation. ILV matrices were characterized; ECM matrices were partly characterized. The ILV validated the method for the quantitation and confirmation analyses of tebuconazole in the first trial for the surface water at LOQ and 10×LOQ and groundwater at 10×LOQ with insignificant modifications to the analytical instruments and parameters. The second validation attempt for groundwater at the LOQ was acceptable using glass jars which had been rinsed with acetonitrile. The ECM should be updated with directives to rinse glass jars with acetonitrile prior to use since this procedure was necessary to achieve acceptable ILV results at the LOQ for groundwater. All ECM and ILV data regarding repeatability, accuracy, precision, and linearity were satisfactory for tebuconazole. ILV specificity data was acceptable for both water matrices (matrix interferences <14% of the LOQ), but ECM specificity data was only acceptable in groundwater. The specificity of the method for surface water was not supported by ECM representative chromatograms since matrix interferences were ca. 30-35% of the LOQ. Based on the overall submitted data, it appeared that carry-over was the problem and not insufficient sample clean-up.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		_					Limit of
	Environmental Chemistry Method	Independent Laboratory Validation	EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Quantitation (LOQ)
Tebuconazole	50755102	50768802		Water ^{1,2}	20/12/2018	Generic Tebuconazole DCI Task Force, c/o United Phosphorus, Inc.	LC/MS/MS	0.100 μg/L

¹ In the ECM, ground water (not characterized) used in the study was filtered well water, prepared by filtering to remove any potential organic contaminants (pp. 11-12 of MRID 50755102). The surface water (pH 6.43, dissolved oxygen concentration 5.8 mg/L) used for this method validation analysis was collected from the Taunton River (SMV Lot No. 14Sep18 Wat-A, collected on 14 September 2018).

I. Principle of the Method

The water sample (5.00 mL) was fortified with 0.0500 or 0.500 mL of 10.0 μ g/L tebuconazole fortification solution (pp. 15-16 of MRID 50755102). Samples were 10x diluted into the calibration range with acetonitrile:purified reagent water (20:80, v:v). Aliquots of the samples were analyzed by LC/MS/MS.

Samples were analyzed for tebuconazole using a Shimadzu LC-20AD HPLC system coupled to an AB MDS Sciex API 4000 mass spectrometer with AB MDS Sciex ESI Turbo V source (pp. 11, 17-18 of MRID 50755102). The LC/MS conditions consisted of a Waters XBridge C18 BEH column (50 x 2.1 mm, 2.5 μ m particle size; column temperature 40°C) with a mobile phase gradient of A) 0.1% formic acid in water and B) 0.1% formic acid in acetonitrile [percent A:B (v:v) at 0.00-0.50 min. 80.0:20.0, 3.00-3.50 min. 0.00:100, 3.51-5.00 min. 80.0:20.0] and MS/MS detection with MRM (source temperature 1.00°C) and positive ESI ionization. Injection volume was 50 μ L. Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: m/z 308.2 \rightarrow 70.2 and 308.2 \rightarrow 125.2 for tebuconazole. Retention time was ca. 2.8 minutes.

In the ILV, the ECM was performed as written, except for insignificant modifications of analytical instruments and parameters (pp. 14, 16-18 of MRID 50768802). A Shimadzu Nexera series HPLC System coupled to an AB Sciex API 5000 Triple Quadrupole mass spectrometer was used. The LC/MS conditions were the same as those of the ECM, except that injection volume was 0.100 μ L for both waters. Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: m/z 308.4 \rightarrow 70.0 and 308.4 \rightarrow 125.0 for tebuconazole. Retention time was ca. 2.5 minutes.

² In the ILV, Fountains Abbey surface water (sample code CS 14/18; pH 7.44, hardness 86 mg/L CaCO $_3$, conductivity 154 μ S/cm, dissolved organic carbon 11.2 mg/L) obtained from The Lake, Studley Royal, Ripon, United Kingdom, and Borehole groundwater (sample code CS 13/18; pH 8.0, hardness 349 mg/L CaCO $_3$, conductivity 436 μ S/cm, dissolved organic carbon 0.00 mg/L) obtained from Smithers Rapra were used (p. 13; Appendix 2, pp. 47-48 of MRID 50768802). Water characterization was performed by Smithers Viscient (ESG) Ltd., Harrogate, United Kingdom.

The Limit of Quantification (LOQ) was $0.100~\mu g/L$ for tebuconazole in water in the ECM and ILV (pp. 19-21 of MRID 50755102; pp. 21-22 of MRID 50768802). In the ECM, the Limit of Detection (LOD) was calculated as 0.0168- $0.0277~\mu g/L$ for groundwater and 0.0539- $0.0583~\mu g/L$ for surface water. In the ILV, the LOD was calculated as 0.0223- $0.0232~\mu g/L$ for Fountains Abbey water and 0.0155- $0.0171~\mu g/L$ for Borehole water. The Method Detection Limit (MDL) was calculated to be $0.05~\mu g/L$ in the ECM and ILV.

II. Recovery Findings

ECM (MRID 50755102): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of tebuconazole in two water matrices at fortification levels of 0.100 μg/L (LOQ) and 1.00 μg/L (10×LOQ; p. 21; Tables 1-4, pp. 27-30). Performance data (recovery results) from primary and confirmatory analyses were comparable, except for the LOQ analysis in groundwater which was only fairly comparable. The ground water (not characterized) used in the study was filtered well water, prepared by filtering to remove any potential organic contaminants (pp. 11-12). The surface water (pH 6.43, dissolved oxygen concentration 5.8 mg/L) used for this method validation analysis was collected from the Taunton River (SMV Lot No. 14Sep18 Wat-A, collected on 14 September 2018).

ILV (MRID 50768802): Mean recoveries and RSDs were within guideline requirements for analysis of tebuconazole in two water matrices at fortification levels of 0.100 µg/L (LOQ) and 50 μg/L (10×LOQ; Tables 1-4, pp. 26-29). Performance data (recovery results) from primary and confirmatory analyses were comparable, except for the LOQ borehole water analysis which was only fairly comparable. Fountains Abbey surface water (sample code CS 14/18; pH 7.44, hardness 86 mg/L CaCO₃, conductivity 154 µS/cm, dissolved organic carbon 11.2 mg/L) obtained from The Lake, Studley Royal, Ripon, United Kingdom, and Borehole groundwater (sample code CS 13/18; pH 8.0, hardness 349 mg/L CaCO₃, conductivity 436 µS/cm, dissolved organic carbon 0.00 mg/L) obtained from Smithers Rapra were used (p. 13; Appendix 2, pp. 47-48). Water characterization was performed by Smithers Viscient (ESG) Ltd., Harrogate, United Kingdom. The ECM method for the quantitation and confirmation analyses of tebuconazole in two water matrices was validated in the first trial for the surface water at LOQ and 10×LOQ and groundwater at 10×LOQ with insignificant modifications to the analytical instruments and parameters (pp. 14, 16-18, 22; Appendix 4, p. 50). The second validation attempt for groundwater at the LOQ was acceptable using glass jars which had been rinsed with acetonitrile. The ECM should be updated with directives to rinse glass jars with acetonitrile prior to use since this procedure was necessary to achieve acceptable ILV results at the LOQ for groundwater.

Table 2. Initial Validation Method Recoveries for Tebuconazole in Water^{1,2}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)		
	Groundwater							
	Quantitation ion							
Tebuconazole	0.100 (LOQ)	5	95.0-101	98.9	2.52	2.55		
	1.00	5	97.3-101	98.6	1.39	1.41		
	Confirmation ion							
Tahaaaaaala	0.100 (LOQ)	5	94.7-117	110	8.75	8.00		
Tebuconazole	1.00	5	92.9-96.4	95.4	1.44	1.50		
	Surface Water							
	Quantitation ion							
Tebuconazole	0.100 (LOQ)	5	92.4-103	97.0	4.56	4.71		
	1.00	4 ³	96.5-101	99.1	1.86	1.88		
	Confirmation ion							
Tebuconazole	0.100 (LOQ)	5	83.0-102	93.8	8.62	9.20		
	1.00	43	96.8-103	99.6	2.51	2.52		

Data (uncorrected recovery results, pp. 19-20) were obtained from p. 21; Tables 1-4, pp. 27-30 of MRID 50755102.

¹ The ground water (not characterized) used in the study was filtered well water, prepared by filtering to remove any potential organic contaminants (pp. 11-12). The surface water (pH 6.43, dissolved oxygen concentration 5.8 mg/L) used for this method validation analysis was collected from the Taunton River (SMV Lot No. 14Sep18 Wat-A, collected on 14 September 2018).

² Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: m/z 308.2 \rightarrow 70.2 and 308.2 \rightarrow 125.2 for tebuconazole.

³ Sample < MDL due to a suspected fortification error (Table 3, p. 29). This sample was considered to be an outlier and is not included in any statistical calculations by the study author. The reviewer could not calculate statistics for n = 5 since the raw data was not included.

Table 3. Independent Validation Method Recoveries for Tebuconazole in Water^{1,2}

Analyte	Fortification	Number	Recovery	Mean	Standard	Relative Standard		
Analyte	Level (µg/L)	of Tests	Range (%)	Recovery (%)	Deviation (%)	Deviation (%)		
	Fountains Abbey Surface Water							
	Quantitation ion							
Tahaaaaaala	0.100 (LOQ)	5	80-98	89	7.1	8.0		
Tebuconazole	1.00	5	92-98	94	2.4	2.5		
	Confirmation ion							
Tahaaaaaala	0.100 (LOQ)	5	81-97	88	6.6	7.6		
Tebuconazole	1.00	5	89-94	92	2.3	2.5		
	Borehole Groundwater							
	Quantitation ion							
Tahaaaaaala	0.100 (LOQ)	5	87-95	91	2.9	3.2		
Tebuconazole	1.00	5	96-107	103	4.3	4.2		
	Confirmation ion							
T-h	0.100 (LOQ)	5	82-88	84	2.4	2.8		
Tebuconazole	1.00	5	97-107	104	3.9	3.8		

Data (uncorrected recovery results, p. 19) were obtained from Tables 1-4, pp. 26-29 of MRID 50768802.

¹ The Fountains Abbey surface water (sample code CS 14/18; pH 7.44, hardness 86 mg/L CaCO₃, conductivity 154 μ S/cm, dissolved organic carbon 11.2 mg/L) obtained from The Lake, Studley Royal, Ripon, United Kingdom, and Borehole groundwater (sample code CS 13/18; pH 8.0, hardness 349 mg/L CaCO₃, conductivity 436 μ S/cm, dissolved organic carbon 0.00 mg/L) obtained from Smithers Rapra were used (p. 13; Appendix 2, pp. 47-48). Water characterization was performed by Smithers Viscient (ESG) Ltd., Harrogate, United Kingdom.

² Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: m/z 308.4 \rightarrow 70.0 and 308.4 \rightarrow 125.0 for tebuconazole. These were similar to those of the ECM.

III. Method Characteristics

The LOQ was $0.100 \,\mu\text{g/L}$ for tebuconazole in water in the ECM and ILV (pp. 19-21 of MRID 50755102; pp. 21-22 of MRID 50768802).

In the ECM and ILV, the LOD was calculated in the ECM using the following equation:

 $LOD = (3x(SN_{ctl}))/(Respls) \times Concls \times DF_{CTRL}$

Where, LOD is the limit of detection of the analysis, SN_{ctl} is the mean signal to noise in height of the control samples (or Blanks), Resp_{LS} is the mean response in height of the two low calibration standards, Conc_{LS} is the concentration of the low calibration standard, and DF_{CTRL} is the dilution factor of the control samples (smallest dilution factor used, i.e. 10.0).

In the ECM, the LOD was calculated as $0.0168\text{-}0.0277~\mu\text{g/L}$ for groundwater and $0.0539\text{-}0.0583~\mu\text{g/L}$ for surface water. In the ILV, the LOD was calculated as $0.0223\text{-}0.0232~\mu\text{g/L}$ for Fountains Abbey water and $0.0155\text{-}0.0171~\mu\text{g/L}$ for Borehole water.

The MDL was calculated using the following equation:

 $MDL = MDL_{LCAL} \times DF_{CTRL}$

Where, MDL is the minimum detection limit, MDL_{LCAL} is the lowest standard concentration (i.e., $0.005 \,\mu g/L$), and DF_{CTRL} is the dilution factor of the control samples (smallest dilution factor used, i.e. 10.0).

The MDL was calculated to be $0.05 \,\mu g/L$ in the ECM and ILV based upon the lowest standard concentration of $0.005 \,\mu g/L$ and a control dilution factor of 10.

Table 4. Method Characteristics

Analyte			Tebuconazole			
Limit of Quantitation (LOQ)	ECM ILV					
Limit of Detection (LOD)	ECM	Groundwater	0.0168 μg/L (Q) 0.0277 μg/L (C)			
		Surface water	0.0539 μg/L (Q) 0.0583 μg/L (C)			
		Surface water	0.0223 μg/L (Q) 0.0232 μg/L (C)			
	ILV	Groundwater	0.0155 μg/L (Q) 0.0171 μg/L (C)			
	ECM	Groundwater	$r^2 = 1.00 (Q)$ $r^2 = 0.999 (C)$			
Linearity		Surface water	$r^2 = 1.00 (Q)$ $r^2 = 0.999 (C)$			
(calibration curve r ²	ILV ¹	Surface water	$r^2 = 0.9990 (Q \& C)$			
and concentration range)		Groundwater	$r^2 = 0.9992 \text{ (Q, LOQ)}$ $r^2 = 0.9982 \text{ (Q, 10×LOQ)}$ $r^2 = 0.9992 \text{ (C, LOQ)}$ $r^2 = 0.9984 \text{ (C, 10×LOQ)}$			
	Range		0.005-0.250 μg/L			
Repeatable	ECM ²		Yes at LOQ and 10×LOQ (two partly characterized water matrices).			
	ILV ^{3,4}		Yes at LOQ and 10×LOQ (two characterized water matrices).			
Reproducible			Yes at LOQ and 10×LOQ.			
Specific	ECM	Groundwater	Yes, matrix interferences were 0-12% of the LOQ (based on peak area); minor baseline noise was observed.			
		Surface water	No, matrix interferences were <i>ca</i> . 30% (Q) and <i>ca</i> . 35% (C) of the LOQ (based on peak area); minor baseline noise was observed. ⁵			
	ILV	Surface water	Yes, matrix interferences were <14% of the LOQ (based on			
		Groundwater	peak area).			

Data were obtained from p. 21 (LOQ/LOD); p. 21; Tables 1-4, pp. 27-30 (recovery data); pp. 14, 22 (correlation coefficients); Figures 1-10, pp. 35-44 (chromatograms); Figures 11-14, pp. 45-48 (calibration curves) of MRID 50755102; p. 22 (LOQ); Tables 1-4, pp. 26-29 (recovery data); p. 21 (linearity); Figures 1-2, p. 33 (calibration curves); Figures 3-20, pp. 34-42 (chromatograms) of MRID 50768802; and DER Attachment 2. Q = Quantitation ion transition; C = Confirmation ion transition.

- 1 ILV correlation coefficients (r²) values were reviewer-calculated from r values provided in the study report (p. 21 of MRID 50768802; DER Attachment 2). In the ECM, solvent-based calibration standards were used for groundwater; matrix-matched standards were used for surface water (pp. 21, 23-24 of MRID 50755102). Solvent-based calibration standards were used for both waters in the ILV since matrix effects were found to be insignificant (<20% difference) for the test waters (pp. 11, 22 of MRID 50768802).
- 2 In the ECM, ground water (not characterized) used in the study was filtered well water, prepared by filtering to remove any potential organic contaminants (pp. 11-12 of MRID 50755102). The surface water (pH 6.43, dissolved oxygen concentration 5.8 mg/L) used for this method validation analysis was collected from the Taunton River (SMV Lot No. 14Sep18 Wat-A, collected on 14 September 2018).
- 3 In the ILV, Fountains Abbey surface water (sample code CS 14/18; pH 7.44, hardness 86 mg/L CaCO $_3$, conductivity 154 μ S/cm, dissolved organic carbon 11.2 mg/L) obtained from The Lake, Studley Royal, Ripon, United Kingdom, and Borehole groundwater (sample code CS 13/18; pH 8.0, hardness 349 mg/L CaCO $_3$, conductivity 436 μ S/cm, dissolved organic carbon 0.00 mg/L) obtained from Smithers Rapra were used (p. 13;

- Appendix 2, pp. 47-48 of MRID 50768802). Water characterization was performed by Smithers Viscient (ESG) Ltd., Harrogate, United Kingdom.
- 4 The ILV validated the ECM method for the quantitation and confirmation analyses of tebuconazole in two water matrices was validated in the first trial for the surface water at LOQ and 10×LOQ and groundwater at 10×LOQ with insignificant modifications to the analytical instruments and parameters (pp. 14, 16-18, 22; Appendix 4, p. 50 of MRID 50768802). The second validation attempt for groundwater at the LOQ was acceptable using glass jars which had been rinsed with acetonitrile. The ECM should be updated with directives to rinse glass jars with acetonitrile prior to use since this procedure was necessary to achieve acceptable ILV results at the LOQ for groundwater.
- 5 Based on Figure 8, p. 42 and Figure 10, p. 44 of MRID 50755102.

IV. Method Deficiencies and Reviewer's Comments

- 1. The ECM should be updated with directives to rinse glass jars with acetonitrile prior to use since this procedure was necessary for the ILV to achieve acceptable results at the LOQ for groundwater (pp. 14, 16-18, 22; Appendix 4, p. 50 of MRID 50768802). The glass jar rinsing was advised by the Sponsor to the ILV after the first ILV validation attempt at LOQ in groundwater failed. The reviewer noted that the acceptable performance data (recovery results) from primary and confirmatory analyses were only fairly comparable for the LOQ borehole water analysis (Tables 1-4, pp. 26-29 of MRID 50768802). The reviewer also noted that the raw data from the failed ILV validation attempt should have been provided for review.
- 2. The specificity of the method for surface water was not supported by ECM representative chromatograms since matrix interferences were *ca*. 30% (Q) and *ca*. 35% (C) of the LOQ (based on peak area; Figure 8, p. 42 and Figure 10, p. 44 of MRID 50755102). In the ECM, the LOD was calculated as 0.0539-0.0583 μg/L for surface water; therefore, these matrix interferences were >50% of the LOD (p. 21). The reviewer also noted that, in the ECM, matrix-matched standards were used for surface water since matrix effects were found to be significant (>20% difference; pp. 21, 23-24).
- 3. The reviewer suspected that there was a carry-over issue occurring during analysis in the ECM and ILV since the ILV remedied their validation issues with rinsing the glass jars and the ECM representative chromatograms showed significant analyte in control samples (Figures 1-10, pp. 35-44 of MRID 50755102; pp. 14, 16-18, 22; Appendix 4, p. 50 of MRID 50768802). Based on the overall submitted data, it appeared that carry-over was the problem and not insufficient sample clean-up.
- 4. The ECM performance data for surface water at 10×LOQ contained only 4 replicates (Table 3, p. 29 of MRID 50755102). The value reported for the fifth replicate was < MDL. The study author suspected fortification error, and this sample was considered to be an outlier and is not included in any statistical calculations by the study author. The reviewer could not calculate statistics for n = 5 since the raw data was not included. All raw data should be submitted and included in statistical data calculations.
- 5. The ECM water matrices were only partly characterized (pp. 11-12 of MRID 50755102).

- 6. The communications of the ILV (Smither Viscient) and Sponsor (Generic Tebuconazole DCI Task Force, c/o United Phosphorus, Inc., and Pyxis Regulatory Consulting, Inc.,) involved the exchange of the definitive protocol, successful completion of the trial, suggestion of rinsing the glass jars with solvent before use, discussion of second attempt, and Sponsor QC check of validation results (pp. 21-22; Appendix 5, p. 51 of MRID 50768802). Technical guidance was provided by the Sponsor to the ILV, but the Sponsor was not the ECM. The reviewer noted that the ECM and ILV laboratories were Smithers Viscient, but the ECM was performed by the Massachusetts location while the ILV was performed by the North Yorkshire location. Reported laboratory personnel differed between the ECM and ILV (p. 5 of MRID 50755102; p. 7 of MRID 50768802).
- 7. The estimation of LOQ in ECM and ILV was not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 19-21 of MRID 50755102; pp. 21-22 of MRID 50768802). In the ECM, the LOQ was defined as the lowest fortification level, and blank values should not be >30% of the LOQ; no calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM. In the ILV, the LOQ was defined as the lowest level validated. The LOD was calculated in the ECM and ILV using the following equation: LOD = (3x(SN_{ctl})/(Resp_{LS}) x Conc_{LS} x DF_{CTRL}, where, LOD is the limit of detection of the analysis, SN_{ctl} is the mean signal to noise in height of the control samples (or Blanks), Resp_{LS} is the mean response in height of the two low calibration standards, Conc_{LS} is the concentration of the low calibration standard, and DF_{CTRL} is the dilution factor of the control samples (smallest dilution factor used, i.e. 10.0). Detection limits should not be based on arbitrary values.

The MDL was calculated to be $0.05~\mu g/L$ in the ECM and ILV based upon the lowest standard concentration of $0.005~\mu g/L$ and a control dilution factor of 10 (pp. 19-21 of MRID 50755102; pp. 21-22 of MRID 50768802).

- 8. In the ECM, solvent-based calibration standards were used for groundwater; matrix-matched standards were used for surface water (pp. 21, 23-24; Tables 5-8, pp. 31-34 of MRID 50755102). Solvent-based calibration standards were used for both waters in the ILV since matrix effects were found to be insignificant (<20% difference) for the test waters (pp. 11, 22; Tables 5-6, pp. 30-31 of MRID 50768802).
- 9. The time required to complete the method for one sample set was not reported in the ILV or ECM.

V. References

- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.
- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures

Tebuconazole (HWG 1608)

IUPAC Name: (RS)-1-p-chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazol-1-

ylmethyl)pentan-3-ol

CAS Name: α -[2-(4-Chlorophenyl)ethyl]- α -(1,1-dimethylethyl)-1H-1,2,4-triazole-1-

ethanol

CAS Number: 107534-96-3

SMILES String: c1cc(Cl)ccc1CCC(O)(C(C)(C)C)Cn2ncnc2